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Endogenous nitrous oxide emission for denitrifiers acclimated with different organic carbons

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Abstract

Nitrous oxide (N_2O) emission occurs during denitrification, such as in biological wastewater treatments, but few studies have focused on N_2O emission during denitrification under endogenous conditions. In the present study denitrifiers were acclimated with acetate or methanol as the electron donor, and endogenous denitrification kinetics including denitrification rates and N_2O emission were examined in batch experiments for the acclimated denitrifiers. Oxidized nitrogen was denitrified slowly during endogenous respiration for denitrifiers acclimated with acetate or methanol, with the denitrification rate of lower than 2 mg/g VSS·h. When the nitrate was used as electron acceptor, a low N_2O emission ratio (lower than 1%) was obtained, while a high emission ratio (above 10%) was observed with nitrite as electron acceptor for both organic carbon acclimated denitrifiers. More attention should be paid to N_2O emission during denitrification under endogenous conditions (such as in the settlement tanks) especially for systems with short-cut biological nitrogen removal through nitrite or systems with a high nitrite accumulation.

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Keywords: Endogenous denitrification; nitrous oxide; denitrification; organic carbon

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1. Introduction

The technologies for biological wastewater treatment has evolved from conventional biological nutrient removal, through enhanced biological nutrient removal to the limit of present technology [1-4]. For sensitive water bodies, discharging of wastewater with total nitrogen (TN) concentration of 3 mg/L and total phosphorus (TP) of 0.1 mg/L or even lower has been set as discharging standard [2-4]. The biological removal of nitrogen (N) is mainly achieved by combining nitrification and denitrification conventional processes. A highly efficient N removal could be achieved through optimized secondary biological nitrogen removal with post nitrogen removal processes. The anaerobic, anoxic and aerobic (A^2O) process has been widely used for biological nitrogen and phosphorus removal from wastewater. Shortages of organic carbon in influent wastewater also occurs, and the supplement of external organic carbons such as acetate and methanol has been examined and practiced for enhancing biological nitrogen removal [5, 6]. Another enhanced biological nitrogen removal is through endogenous denitrification in intermittently aerated systems or in processes with alternative anoxic/oxic phases [7, 8].

During denitrification, the emission of nitrous oxide (N_2O) may occur. N_2O is one type of greenhouse gas, with a global warming potential of around 300 times that of carbon dioxide (CO_2) and a life-cycle of 114 years [9]. The effect of factors such as organic carbon (type and concentration), carbon/nitrogen (C/N) ratios, nitrite (NO_2^--N) concentration, dissolved oxygen (DO) concentration and others on the denitrifying activities and N_2O emissions have been examined, while less study has been focused on N_2O emission during endogenous denitrification [10-13]. During denitrification a high N_2O emission potential existed with intracellular organic carbons (such as polyhydroxybutyrate, PHB) as the electron donor due to its slow degradation rate [14]. Therefore, we proposed that denitrification during endogenous conditions might enhance N_2O emission due to its even much low denitrification rate. Nitrite is one important factor inducing N_2O emission during denitrification through inhibiting N_2O reductase [12]. Denitrifiers with different metabolic modes could be acclimated with different organic carbons. For example, when acetate was used as the carbon substrate, *Comamonadaceae* and *Rhodocyclaceae* from the *Beta-proteobacteria* group, and *Rhodobacteraceae* from the *Alpha-proteobacteria* group were detected [15, 16]. While *Methylaphilaceae* and *Hyphomicrobiaceae* were detected when methanol was used as the carbon substrate [16]. Different types of denitrifiers might posses different denitrification mode under endogenous conditions.

In this study, denitrifiers were acclimated with acetate or methanol as the electron donor, and then endogenous denitrification kinetics including denitrification rates and N_2O emission were examined in batch experiments for the acclimated denitrifiers.

2. Materials and Methods

2.1 Denitrifying activated sludge acclimation

Two sequencing batch reactors (SBR_A with acetate as the electron donor and SBR_M with methanol as the electron donor), each with a working volume of 5.4 L, were operated at 25°C to enrich denitrifiers. The SBRs were operated six cycles per day and each cycle comprised phases of fill (20 min), anoxic (150 min), aerobic (10 min), settlement (40 min) and draw/idle (20 min). The reactors were mixed during the fill and anoxic phase by mechanical mixers. The aerobic phase was adopted to remove nitrogen gas (N_2) produced during the anoxic phase so as to improve the settlement properties of activated sludge. In each cycle, 1.8 L of treated wastewater was exchanged with a new batch of synthetic wastewater and a hydraulic retention time (HRT) of 12 hours was used. Sludge was removed once a day from the reactor before the settlement phase to maintain a sludge retention time (SRT) of around 10 days.

The SBRs were fed with synthetic wastewater with components of 510 mg/L sodium acetate or 0.34 mL/L methanol, 607 mg/L $NaNO_3$, 10 mg/L yeast extract, 76 mg/L NH_4Cl, 25 mg/L Na_2HPO_4, 90 mg/L $MgSO_4 \cdot 7H_2O$, 14 mg/L $CaCl_2 \cdot 2H_2O$, and 1 mL trace elements. Trace elements were added following Smolders *et al.* [17]. The reactors were seeded with activated sludge taken from Nanshan Wastewater Treatment Plant, Shenzhen, China.

2.2 Batch experiments

Endogenous denitrification with electron acceptors of only nitrate (NO_3^--N), only NO_2^--N and NO_3^--N plus NO_2^--N was carried out in batch reactors for denitrifiers acclimated with acetate or methanol. The batch reactors were made from 500 mL capped glass flasks, each with three ports on the cap, one for liquid sampling, one for gas sampling, and the other for gas balance.

Activated sludge mixed liquor was withdrawn from the parent SBR_A and SBR_M at the end of the aerobic phase. After withdrawal from the reactor, the mixed liquor was centrifuged and the remaining sludge was re-suspended in a solution as those of the synthetic wastewater but without the addition of electron donors and electron acceptors. Stock NO_3^--N or NO_2^--N solutions were added to the batch reactors to achieve an initial NO_3^--N concentration of 100 mg/L, the initial NO_2^--N concentration of 50 mg/L, and the initial NO_3^--N of 100 mg/L and NO_2^--N of 20 mg/L, respectively. The batch reactors were then capped and mixed with magnetic stirrers at 25°C. Samples were then taken at intervals from the batch reactors to test PHB (for acetate as the organic carbon), NO_2^--N and NO_3^--N for liquid samples, and N_2O for gas samples.

2.3 Analytical methods

NO_2^--N and NO_3^--N were analyzed by an ICS-1500 ion chromatography (Dionex, USA). Suspended solids (SS) and volatile suspended solids (VSS) were determined according to Standard Methods [18].

The PHB concentration was detected by the modified high performance liquid chromatography (HPLC) method [19, 20]. The HPLC (LC-10A, Shimadzu, Japan) with a UV index detector and an Aminex HPX-87H column (Bio-Rad, USA) was used. Separation during HPLC tests was achieved using a mobile phase of 1‰ (vol/vol) H_2SO_4 at a flow rate of 0.6 mL/min, a column temperature of 35°C, and a detector temperature of 40°C, and crotonic acid was used for calibration.

N_2O was detected by a gas chromatography (GC, Agilent 6820, Agilent Technologies, USA) with an electron capture detector (ECD) and a HP-PLOT/Q column (J&W GC Columns, Agilent Technologies, USA). Temperatures during testing were 50°C for the injection port, 50°C for the oven, and 300°C for the detector. Nitrogen gas was used as the carrier gas at a flow rate of 15 mL/min. N_2O gas was used as the standard for calibration. For convenient comparison, the produced N_2O in the gas phase was expressed as mg/L, representing mg N_2O (gas) produced from the specific volume (L) of mixed activated sludge liquor.

3. Results and Discussion

The two laboratory-scale SBRs were operated for more than 6 months and denitrifiers were successfully enriched with acetate or methanol as the organic carbon. As to the NO_3^--N concentration of around 100 mg/L in the feeding wastewater, it was reduced to less than 1 mg/L in the treated effluent and only a small amount of NO_2^--N was detected in the effluent. During the batch experiments, endogenous denitrification kinetics and N_2O emission under various electron acceptor conditions for denitrifiers acclimated with acetate or methanol is shown in Figure 1 and the regressed denitrification rates is given in Table 1.

Under all conditions, oxidized nitrogen was denitrified slowly during endogenous respiration. For denitrifiers acclimated with acetate, there was a small amount of PHB remaining inside the biomass and PHB was also utilized slowly for respiration. When NO_3^--N or NO_3^--N+NO_2^--N was used as the electron acceptor, accumulation of nitrite was observed under all conditions, indicating a high NO_3^--N reduction rate than the NO_2^--N reduction rate. The reason could be due to that denitrifiers prefer NO_3^--N rather than NO_2^--N under endogenous respiration conditions with limited supply of organic carbons. With NO_3^--N as the electron acceptor, the ratio of produced NO_2^--N to the reduced NO_3^--N ratio was 53% for acetate acclimated denitrifiers and it was 38% for methanol acclimated denitrifiers; when both NO_3^--N and NO_2^--N as electron acceptors, the ratio was 66% for acetate acclimated denitrifiers and it was 28% for methanol acclimated denitrifiers. This showed that a high potential of nitrite accumulation for acetate acclimated denitrifiers during denitrification. The partial utilization of PHB might

contribute to this phenomenon, because a high nitrite accumulation often occurred with PHB as the organic carbon for denitrification [14].

The denitrificaion rate was relatively low during endogenous respiration. The net removal of oxidized nitrogen was less than 1 mg/g VSS·h for both acetate or methanol acclimated denitrifiers. However, a slightly higher NO_3^--N reduction rate occurred for acetate acclimated denitrifiers than that of methanol acclimated denitrifiers. Still, we have to remember that a high accumulation of nitrite occurred with acetate acclimated denitrifiers. Therefore, due to the low endogenous denitrification rate, as to post-denitrification with endogenous respiration for nitrogen removal, a long reaction time or a high concentration of biomass should be maintained to achieve the expected denitrification efficiency. Therefore, processes such as biofilm systems or membrane bioreactors would be a better choice for nitrogen removal using endogenous denitrification.

Table 1. Endogenous denitrification rates (mg/g VSS·h) for denitrifiers acclimated with acetate or methanol as the organic carbon. The minus indicates the reduction rate and the others indicate the production rate.

Organic carbon	Electron acceptor / Parameters	NO_3^--N	NO_2^--N+ NO_3^--N	NO_2^--N
Acetate	NO_3^--N	-1.976	-1.425	-
	NO_2^--N	1.048	0.943	-0.849
	NOx-N	-0.928	-0.482	-0.849
	N_2O	0.026	0.079	0.157
	PHB	-1.027	-0.377	-0.362
Methanol	NO_3^--N	-0.846	-1.08	-
	NO_2^--N	0.324	0.2994	-0.492
	NOx-N	-0.374	-0.560	-0.353
	N_2O	0.0033	0.0582	0.108

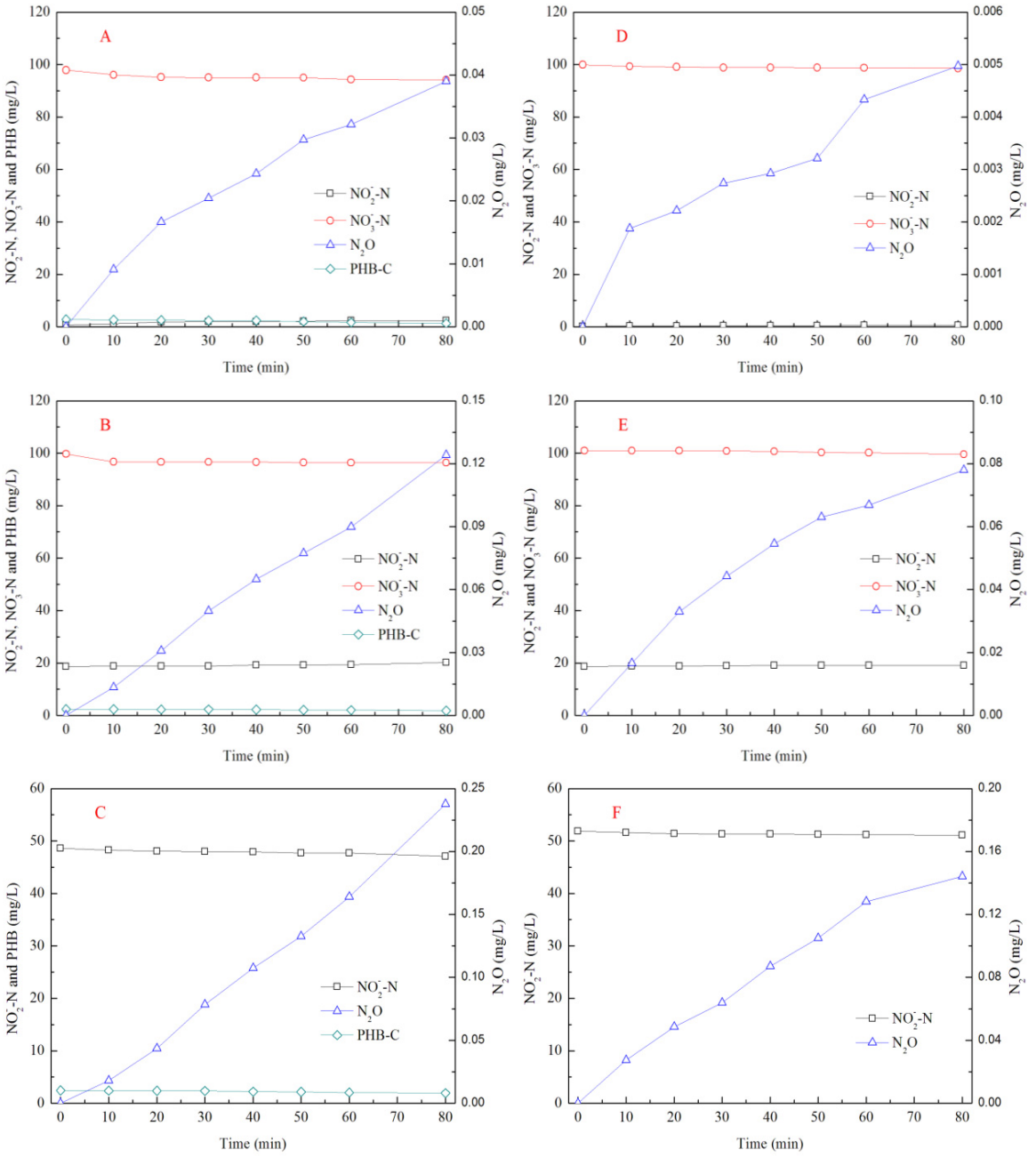

Fig. 1. N_2O emission during endogenous denitrification for denitrifiers acclimated with acetate (A, B and C) or methanol (D, E and F) as the organic carbon. A and D: only NO_3^--N; B and E: NO_2^--N+NO_3^--N; C and F: only NO_2^--N.

N_2O emission occurred under all endogenous denitrification conditions. With acetate acclimated denitrifiers, the N_2O emission ratio to the removed NO_3^--N or NO_2^--N was 0.8% for NO_3-N as the electron acceptor, 3.5% for NO_3^--N and NO_2^--N as the electron acceptor, and 11.8% for NO_2^--N as the electron acceptor. These ratios were 0.25%, 3.4% and 14.0% for methanol acclimated denitrifiers, respectively. With respect to the removed NOx-N, these ratios were 1.8%, 10.4% and 11.8% for acetate acclimated denitrifiers, respectively, and 0.4%, 4.7% and 14.0% for methanol acclimated denitrifiers, respectively. With NO_3^--N as the electron acceptor, a relatively low N_2O emission ratio (lower than 1%) was obtained, while a very high emission ratio (above 10%) was observed with NO_2^--N as the electron acceptor for both organic carbon acclimated denitrifiers. Nitrite existence always promoted N_2O emission both during nitrification and denitrification processes. During denitrification, N_2O emission was mainly due to diffusion from the liquid to the gas phase and there was no stripping like during aerobic conditions. Therefore, the emission of N_2O would be limited and N_2O generation during denitrification might be much higher than those obtained by testing the gas samples. Based on the above results N_2O emission potential under endogenous denitrification conditions (such as in the settlement tanks) might be very high and more attention should be paid to this, especially for systems with a high nitrite accumulation, such as short-cut biological nitrogen removal processes.

4. Conclusions

The following conclusions could be obtained: (i) oxidized nitrogen was denitrified slowly during endogenous respiration for both denitrifiers acclimated with acetate or methanol, with the denitrification rate of lower than 2 mg/g VSS·h; (ii) with NO_3^--N as the electron acceptor, a relatively low N_2O emission ratio (lower than 1%) was obtained, while a very high emission ratio (above 10%) was observed with NO_2^--N as the electron acceptor for both organic carbon acclimated denitrifiers.

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